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Higher flight activity in the offspring of migrants compared to residents in a migratory insect

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1 **Higher flight activity in the offspring of migrants compared to residents in a migratory**
2 **insect**

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17

18 **Abstract**

19

20 Migration has evolved among many animal taxa and migratory species are found across all
21 major lineages. Insects are the most abundant and diverse terrestrial migrants, with trillions of
22 animals migrating annually. Partial migration, where populations consist of resident and
23 migratory individuals, is ubiquitous among many taxa. However, the underlying mechanisms
24 are relatively poorly understood and may be driven by physiological, behavioural or genetic
25 variation within populations. We investigated the differences in migratory tendency between
26 migratory and resident phenotypes of the hoverfly, *Episyrphus balteatus*, using tethered flight
27 mills. Further, to test whether migratory flight behaviour is heritable, we compared the flight
28 behaviour of laboratory-reared offspring of migrating, overwintering and summer animals.
29 Offspring of migrants attempted more flights than resident individuals. Interestingly, there
30 were no differences among wild-caught phenotypes with regard to number of flights or total
31 flight duration. Low activity in field-collected migrants might be explained by an energy
32 conserving state that migrants enter in to when under laboratory conditions. Our results
33 strongly suggest that flight behaviour is heritable and that genetic factors influence migratory
34 tendency in *E. balteatus*. These findings support the growing evidence that genetic factors
35 might play a role in partial migration and warrants careful further investigation.

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37 **Keywords:** flight behaviour, heritability, migratory restlessness, partial migration, tethered
38 flight mill.

1. Introduction

Migration has evolved independently among many animal taxa, and migrating animals comprise a large proportion of all major lineages [1-4], with insects being the most abundant and speciose terrestrial migrants [5-7]. The most common type of migration is partial migration, which is defined by variation in migratory tendency within species [8-10]. Hence, partially migratory populations are composed of a mixture of resident and migratory individuals simultaneously [8,9]. Many examples of partially migratory species have been reported in mammals, birds, fish and invertebrates [8].

As natural selection acts upon individuals, it is important to determine the underlying mechanisms driving differences in individual migratory tendency [8]. Individual differences in migratory tendency between animals of the same population may underpin the extent of partial migration observed within species [8,11]. Possible drivers of differences in migratory tendency can be heterogeneity within populations, such as physiological, morphological, behavioural or genetic variation [8]. For example, in birds, morphological variation has been shown to influence migratory tendency, with a smaller body size usually promoting migratory behaviour [12]. Furthermore, behavioural differences driving migratory tendency have been found in the fish *Rutilus rutilus*, where bold individuals are more likely to migrate [11], and in insects [13]. This provides evidence for a strong behavioural component influencing partial migration and suggests that differences in activity between individuals might influence migratory tendency or variation in dispersal ability in insects [14].

Migratory behaviour has been shown to be heritable in a number of animal taxa. The blackcap, *Sylvia atricapilla*, is a particularly well studied example, where migratory activity and the behaviour associated with it, known as migratory restlessness, is strongly heritable

[e.g. 15,16]. The heritability of flight behaviour is also known in insects. For example, migratory tendency has been shown to be heritable in the moths *Spodoptera exempta*, *Mythimna separata*, *Helicoverpa armigera*, and the grasshopper *Melanoplus sanguinipes* [17-20]. However, in other species such as *Locusta migratoria* and *Schistocerca gregaria*, migratory behaviour is strongly influenced by environmental factors [19]. Therefore, we may predict that behavioural differences and the heritability of behavioural traits may play an important role in driving levels of partial migration within populations.

In Europe, some hoverfly species (Diptera, Syrphidae) are partially migratory, where part of the population overwinters in the breeding grounds as adults, while others travel large distances in search of a milder climate [13,21,22]. *Episyrphus balteatus* is the most common migrant hoverfly in Europe and during winter a part of the population remains in the habitat and overwinters as larvae, pupae or adults [23-26], whereas other individuals of the population migrate south to the Mediterranean in autumn, where they breed throughout the winter [27,28]. *Episyrphus balteatus* is an important pollinator and the larvae are efficient aphid predators, playing a significant role in the biocontrol of agricultural crop pests [29-31]. Adult overwintering hoverflies are almost exclusively females that are in a facultative reproductive diapause, whereas males of *E. balteatus* are thought to be unable to increase their fat bodies and therefore are more susceptible to cold temperatures and are not expected to overwinter [32,33]. Most studies so far have focused on the southward flights to the Mediterranean in autumn [22,27,34]. Females migrate with an undeveloped reproductive system, but sperm storage organs already full of sperm [33]. Currently, there is no description of the northward flight back to central and northern Europe in spring [28,35]. The short life-span of this species indicates that the migration system of *E. balteatus* is multi-generational, with a single generation moving south in the autumn and successive generations moving north in the spring [13,28,35], as is typical of many latitudinal insect migrations [5]. Interestingly,

no genetic differentiation has yet been found between different overwintering strategies of *E. balteatus* [36], with very low genetic distances between populations and a lack of population subdivision [37]. Therefore, it is thought all individuals may have the genetic material for the expression of the different overwintering phenotypes and that their decision for one of the strategies may depend on environmental and individual factors [36]. To date it is unclear whether environmental or genetic factors are responsible for the decision of individuals to migrate, or whether it is a combination of both.

Behavioural traits, such as the propensity to engage in long-distance flight and flight tendency are crucial proxies for migratory potential or individual migratory tendency, and can be quantified using tethered flight mills under controlled conditions [14]. Tethered flight trials are a good way to measure flight behaviour and have been used to investigate flight potential in a number of insect species [e.g. 14,18,38-41]. In this study, we investigated the migratory tendency between different migratory phenotypes of *E. balteatus* using tethered flight mills. In order to disentangle environmental effects that possibly trigger migration, such as weather and food resources, we investigated differences in the flight behaviour of first generation hoverflies deriving from overwintering, migrating and summer populations. Furthermore, to investigate the importance of environmental effects, wild caught hoverflies from both migrating and overwintering populations were also tested. Specifically, we aimed to answer the following questions: (i) Do first generation hoverflies deriving from summer populations, migrating or overwintering hoverflies differ in their flight behaviour? (ii) Do female and male hoverflies descending from the different phenotypes differ in their flight behaviour? (iii) Do female and male hoverflies captured during migration differ in their flight behaviour?

We expect individuals deriving from migrating populations to attempt more flights and spend more time flying on the tethered flight mills than individuals deriving from overwintering or

summer populations. Moreover, we expect migrating and overwintering individuals collected in the field to differ in their flight behaviour; with migrating animals showing a stronger tendency to fly and a longer duration of flight. Since females have been observed in significantly larger numbers while migrating, they are expected to attempt more flights and spend more time flying on the mill than males.

2. Methods

(a) Study animals

Hoverflies for flight mill experiments were divided into three phenotypes: Resident overwintering, resident summer and migratory. Resident overwintering and summer hoverflies were caught in the surroundings of Bern, Switzerland (46°56'38"N, 7°26'49"E) from April to November 2016 on sunny and warm days (figure S1). Individuals caught in April were assigned to the overwintering phenotype, since only females were found during this period of time. Males would indicate the possible return of migrants, as they do not normally overwinter [33]. Additional overwintering flies were caught in November. Because of harsh conditions in the beginning of November, migrants are believed to have started migrating south already. Flies designated as summer individuals were caught in June and July, in the same locations as overwintering flies. Migrating flies were captured at Col de Bretolet (46°08'34.1"N 6°47'45.2"E), an alpine pass at 1923 m a.s.l. on the border between Switzerland and France, in September and October 2016 (figure S1). Migrating flies were caught during active migration, heading southwest over the pass in large numbers.

Hoverflies were put into flight cages (45 x 45 x 90 cm) that consisted of a white plastic frame covered by nylon gauze in a climate chamber at 20°C, with a day-night cycle of 16:8 h

light:dark. Flies were kept in groups of up to 20 individuals per cage. Each cage had a layer of kitchen paper on the ground. One petri dish with moist cotton wool served as water supply and an additional petri dish contained cotton wool with sugar water (10% sugar) and some mashed pollen. Additionally, flies were provided with a small ball of pollen, powdered sugar and honey (60% pollen, 30% powder sugar and 10% honey). All petri dishes were checked and refilled daily.

(b) Rearing of *E. balteatus* in the laboratory

Hoverflies were reared in a climate chamber at 20°C with a day-night cycle of 16:8 h light:dark. A bean plant (*Vicia faba*) infested with aphids (*Acyrtosiphon pisum*) was placed into the cage on the same day the hoverflies were caught, to stimulate egg laying. Bean plants were checked for eggs and larvae daily. Larvae were placed individually in vials with a bean leaf for shelter. Larvae were fed between 20-30 aphids daily. When pupated, the leaf and the leftover aphids were taken out of the vial to increase the chance of a smooth emergence [24,42]. Once emerged, the flies were put into a flight cage (45 x 45 x 90 cm) for between 12 and 24 hours, where they were given the possibility to feed and fly before the start of the experiments.

(c) Tethered flight mill experiments

Tethered flight mills were used to investigate the flight behaviour of individual *E. balteatus* (figure 1). Flight mills were designed at Rothamsted Research (Patent: [43]) and consist of a lightweight wire arm suspended between two magnets, which results in almost no resistance against the turning of the arm. This means that even weak fliers are able to turn the mill and fly rotationally in a horizontal plane (see also [14]). The hoverfly was glued to a pin, attached

to one end of the arm of the mill, using a contact adhesive. A striped disc attached to the axis turns with the arm (figure 1). A light sensor detects the movement of the disc and records the distance flown (m), time spent flying (s) and flight speed (m/s). This data is used to calculate different measurements of distance, duration and speed of specific flights, e.g. the furthest flight or the first flight. The system used has five channels, allowing five individual insects to be flown simultaneously. Data for each individual was processed using a custom-written script (K.S.L.) in Matlab (The MathWorks Inc. 2012, see also [14,40] for further details on the flight mills). Representative outputs from the flight mills are presented in figure S2.

Flight experiments were conducted between May and November 2016. Flies with damaged or missing wings, or that were no longer able to fly were not used for the experiments. Before gluing the hoverfly to the pin, a flight test was conducted by using a vial and releasing the fly in to the air. If a hoverfly was not able to fly, it was excluded from any further experiments. All individuals were randomly assigned to one of the 5 flight mills. Hoverflies were flown in a clockwise rotation. Immediately after putting the flies on the flight mills, they were given a piece of paper (approximately 1 x 1 cm) as a platform. At the start of the experiments, the flight mill recording program was started and the piece of paper was removed as simultaneously as possible from all flies. Hoverflies that did not start flying were stimulated to fly once by putting a finger under their body and then removing it. During the experiments, the flies were able to see each other, therefore, experiments were only conducted when at least two individuals were present. All individuals were left on the flight mill for 4 hours, regardless of their flight behaviour. Any hoverfly that looked damaged, unhealthy or had died during the experiment was excluded from further analysis (5 of 232 animals tested). Observational experiments were also carried out to distinguish flight from hovering: hovers never resulted in the striped disc of the flight mill moving for more than 10 seconds, and thus

flights of less than 10 sec were excluded. Immediately after the flight mill experiments, hoverflies were stored separately in a freezer (-25 °C).

To investigate differences in flight ability and behaviour between wild caught individuals, a subset of migratory individuals and all overwintering individuals captured in November were used for tethered flight mill experiments directly. In this case, flies were given a minimum of 12 hours to acclimatize to the conditions in the climate chambers before the start of the experiments. The experiment was started within the first 72 hours after capture. All laboratory-reared individuals were flown within the first day after eclosion to ensure that they did not already show reproductive behaviour [17].

(d) Statistical Analysis

All statistical analyses were conducted using R version 3.3.2 [44]. Initially, a generalised linear model with a binomial error distribution was used to compare flight ability between the first generation offspring of the different overwintering phenotypes. Sex and the interaction between sex and phenotype were incorporated as fixed effects in the model. The same procedure was applied to compare flight ability between wild caught overwintering and migrating flies and for comparing the two treatments (wild caught and lab reared) within sex and within phenotype (see Supplementary Material).

For further analysis, only data from flies that had flown at least two flights over 1 m/s were included to ensure that we only analysed individuals that were able to fly on the mill. A correlation test was performed to determine the relationship between the variables recorded with the flight mills (figure S3). Three parameters considered important for migration were initially chosen, total duration of flights, total distance flown and number of flights. As total

distance and total duration were correlated, further analyses were conducted using total duration and number of flights. These two variables were log-transformed, so that the residuals of the models visually indicated a normal distribution.

Total flight duration and number of flights were compared between first generation offspring from the different overwintering phenotypes, using a binomial linear model. Sex and the interaction between sex and phenotype were included as fixed effects in the model, since there is expected to be a difference in flight behaviour between sexes. The same procedure was done for the comparison of the different wild caught phenotypes. *p*-values were extracted from the models by re-levelling of the intercept. Furthermore, male and female hoverflies within the migratory phenotype were compared to each other using a linear model with sex as a fixed effect.

3. Results

Overall, 72% of all individuals (168 of 232) were able to fly on the flight mills. There were no significant differences in flight ability between the different phenotypes of the lab reared animals, and between the two phenotypes collected in the field. Flight ability within phenotype and within sex between wild caught and lab reared individuals was also not significantly different (all $p \geq 0.353$, see Supplementary Material).

In both sexes, there was a trend for offspring of migrants (females: $n = 13$, males: $n = 13$) to initiate more flights than offspring of overwintering females (females: $n = 23$, $p = 0.0497$; males: $n = 23$, $p = 0.099$) and offspring of summer females (females: $n = 31$, $p = 0.026$; males: $n = 31$, $p = 0.045$) (table 1, figure 2a). By contrast, the number of flights did not differ between offspring of overwintering and summer females ($p > 0.05$ for males and females),

nor did it differ between the sexes in any of the phenotypes ($p > 0.05$ in all cases; table 1, figure 2a).

Although the offspring of migrants initiated more flights than the offspring of the other phenotypes, this increase did not typically translate into longer total flight duration. Only in the case of male offspring from migrants compared with male offspring from summer flies was there a significant increase in flight duration ($p = 0.044$; table 1, figure 2b). All other pair-wise comparisons of phenotypes were non-significant, nor did flight duration differ between the sexes in any of the phenotypes ($p > 0.05$ in all cases; table 1, figure 2a).

The trend for increased flight activity observed in the offspring of migrants compared to other phenotypes was not supported by the comparison of wild-caught migrants (females: $n = 22$, males: $n = 6$) and overwintering flies (females: $n = 8$, males: $n = 14$). In both sexes, number of flights (figure 3a) and total flight duration (figure 3b) were not significantly different from each other ($p > 0.05$ in all cases; table 2). In migrants, males had significantly longer total flight durations than females ($p = 0.041$; figure 3b), but sexes did not differ in their flight activity in the wintering flies. Comparison between wild caught and lab reared hoverflies within phenotype and within sex showed no significant differences in the total number of flights, nor total flight duration (table 3).

4. Discussion

We have demonstrated behavioural differences between the offspring of resident and migrating *E. balteatus*, with the offspring of migrants undertaking more flights than the offspring of summer or overwintering animals. Interestingly, we found no difference in flight behaviour between actively migrating and overwintering hoverflies collected from the field,

under laboratory conditions. However, actively migrating males differed significantly in the total duration of flights from migrating females, whereas there was no difference between the sexes of migrant offspring. There were no differences observed in the flight ability on the mill between sexes or phenotypes.

We observed behavioural differences between the offspring of hoverflies from different overwintering strategies, suggesting that the decision to migrate in hoverflies might be heritable. While Raymond *et al.* [36] did not detect any genetic differentiation between the different overwintering strategies, using microsatellites, such differentiation might not be detected using neutral markers [45]. First generation migrants initiated more flights throughout the experiment than offspring of overwintering hoverflies. Heritability of migratory tendency has been found in several species of insects, such as the moths *Mythimna separata* [18] and *Spodoptera exempta* [20], and the grasshopper *Melanoplus sanguinipes* [19], but this is the first investigation of this phenomenon in hoverflies. Interestingly, no significant difference was detected in total flight duration between the offspring of migrating and overwintering flies. It is believed that environmental cues such as wind [46-48] or a change in temperature [49] are important for the departure or the continuation of migratory movement in insects [5]. Hoverflies did not receive these environmental cues in the laboratory, as temperature stayed constant throughout the experiment, and wind in the laboratory was not possible, since flight mills record even the slightest movement. These factors, or the absence of them, might be the reason why the short flights did not turn into longer migratory flights. However, the increased number of attempts to fly in the offspring of migrants suggests that they are more prone to flying than the offspring of other phenotypes. Heritability in the urge to fly, also called migratory restlessness, is relatively well explored in birds [e.g. 16,50-52]. Here, the number of attempted flights in *E. balteatus* could be

considered as a comparable assay to migratory restlessness, and may prove useful for determining migratory propensity in hoverflies and other migratory insects.

No significant difference was detected between wild caught migrants and overwintering individuals in number of flights or total flight duration. We believe that this relative lack of activity is due to an energy conserving state these migrating animals fall into, when caught out of active migration and transported to the laboratory. Similar results were found by Odermatt *et al.* [13], who showed that migratory flies tended to be less active than summer individuals, when tested in the laboratory. Migratory flights have been shown to involve immense energy expenditures in birds [49] and this is also true for insects [53,54]. Thus, we may expect physiological differences between migratory and resident phenotypes, as shown by Attisano *et al.* [38] in milkweed bugs, *Oncopeltus fasciatus*. Another reason for the lack of difference in flight behaviour between wild caught migratory and overwintering hoverflies could be due to the absence of certain environmental cues in the laboratory. Similarly to triggering migration, individuals collected from the field might depend on these environmental cues for the continuation of their migratory flight. Since in the laboratory, they are not exposed to these cues, they may conserve their energy for when they are able to continue their migration [13].

Furthermore we found a difference in total flight duration between wild caught male and female migrants, with male migrants undertaking significantly longer flights than females. Male migrants do not have any reproductive constraints, whereas females migrate with an undeveloped reproductive system [33]. During migration, females reallocate their energy away from reproduction, but still experience reproductive constraints by maintaining sperm storage organs [33]. This might explain the longer flight duration in males compared to females. While no differences were shown in resting metabolic rate between male and female *E. balteatus* [55], this is yet to be investigated in migrating individuals. No significant

difference in flight duration was observed between male and female offspring of migrants. This might be explained by the offspring being freshly emerged and a maximum of 48 hours old. Reproduction is not important in the first few days, and females only start laying eggs after 12 days [56]. The number of flights was not significantly different between male and female migrants and neither between male and female migrant offspring. However, this is what we expect. Male and female hoverflies both migrate, although males are the less abundant sex during migration [57]. Therefore, they were not expected to differ in their propensity to fly. The number of flights is less restricted by the difference in energy expenditure of the two sexes, since most of the energy is used during long flights.

In summary, we have shown that offspring of different phenotypes differ in their flight behaviour, strongly suggesting genetic factors influencing migratory tendency in *E. balteatus*. Moreover, we hypothesize that environmental cues do remain important for migration, as we found no difference in flight behaviour between the different wild caught phenotypes. Furthermore, we have shown behavioural differences between sexes within the migratory phenotype, supporting the evidence for reproductive constraints of migrating females [33]. Future investigations should include studies under field conditions that are close to conditions hoverflies experience during migration. Thereby, environmental factors could be included into tethered flight mill experiments.

Data accessibility. Data are available from the Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.44hc2>) [58].

Author's contributions. L.J.D., A.G., K.S.L., J.W.C., and M.H.M.M designed the experiments. L.J.D. and A.G. carried out the experiments with assistance from K.S.L. and

M.H.M.M. L.J.D. wrote the initial draft of the manuscript with assistance from M.H.M.M., and input and revisions from all authors.

Competing interests. The authors have no competing interests.

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516

Table 1. Estimates from linear models comparing number of flights and total duration between first generation females (F) and males (M) of different phenotypes.

Comparison	Estimate	SE	<i>P</i>
Number of flights (log)			
Migrating M vs. Overwintering M	-0.546	0.329	0.099
Migrating M vs. Summer M	-0.651	0.322	0.045
Overwintering M vs. Summer M	-0.105	0.271	0.700
Migrating F vs. Overwintering F	-0.652	0.329	0.049
Migrating F vs. Summer F	-0.759	0.337	0.026
Overwintering F vs. Summer F	-0.107	0.290	0.712
Migrating M vs. Migrating F	-0.308	0.372	0.408
Overwintering M vs. Overwintering F	-0.414	0.279	0.141
Summer M vs. Summer F	-0.417	0.282	0.142
Total duration (log)			
Migrating M vs. Overwintering M	-0.396	0.361	0.275
Migrating M vs. Summer M	-0.722	0.354	0.044
Overwintering M vs. Summer M	-0.326	0.298	0.277
Migrating F vs. Overwintering F	-0.201	0.361	0.580
Migrating F vs. Summer F	-0.265	0.371	0.477
Overwintering F vs. Summer F	-0.064	0.318	0.841
Migrating M vs. Migrating F	-0.412	0.409	0.315
Overwintering M vs. Overwintering F	-0.217	0.307	0.482
Summer M vs. Summer F	0.044	0.310	0.886

Significant differences ($P < 0.05$) are presented in bold. Transformations are presented in parentheses.

Table 2. Estimates from linear models comparing number of flights and total duration between females (F) and males (M) of different wild caught phenotypes.

Comparison	Estimate	SE	<i>P</i>
Number of flights (log)			
Migrating M vs. Overwintering M	-0.544	0.483	0.266
Migrating F vs. Overwintering F	0.146	0.408	0.722
Migrating M vs. Migrating F	-0.752	0.456	0.106
Overwintering M vs. Overwintering F	-0.062	0.438	0.887
Total duration (log)			
Migrating M vs. Overwintering M	-0.860	0.496	0.053
Migrating F vs. Overwintering F	0.191	0.419	0.650
Migrating M vs. Migrating F	-0.985	0.468	0.041
Overwintering M vs. Overwintering F	0.193	0.450	0.671

Significant differences ($P < 0.05$) are presented in bold. Transformations are presented in parentheses.

Table 3. Estimates from linear models comparing number of flights and total duration between wild caught and lab reared hoverflies within the same phenotype.

Comparison	Estimate	SE	<i>P</i>
Number of flights (log)			
Migrating M wild vs. Migrating M lab	-0.391	0.441	0.380
Migrating F wild vs. Migrating F lab	0.053	0.312	0.866
Overwintering M wild vs. Overwintering M lab	-0.393	0.363	0.282
Overwintering F wild vs. Overwintering F lab	-0.745	0.439	0.095
Total duration (log)			
Migrating M wild vs. Migrating M lab	-0.430	0.487	0.381
Migrating F wild vs. Migrating F lab	0.143	0.345	0.681
Overwintering M wild vs. Overwintering M lab	0.160	0.369	0.666
Overwintering F wild vs. Overwintering F lab	-0.249	0.447	0.579

Significant differences ($P < 0.05$) are presented in bold. Transformations are presented in parentheses.

Figure captions

Figure 1. Tethered flight mill. Labelled photograph of an individual flight mill (*a*) and a close up of *Episyrphus balteatus* attached to the flight mill (*b*).

Figure 2. Number of flights (*a*) and flight duration in seconds (*b*) between phenotypes and sexes of first generation hoverflies. For clarification, only significant *p*-values are indicated in the graph. $*p < 0.05$. Number of flights and flight duration have been log transformed.

Figure 3. Number of flights (*a*) and total flight duration in seconds (*b*) between wild caught migrating and overwintering hoverflies. For clarification, only significant *p*-values are indicated in the graph. $*p < 0.05$. Number of flights and flight duration have been log transformed.

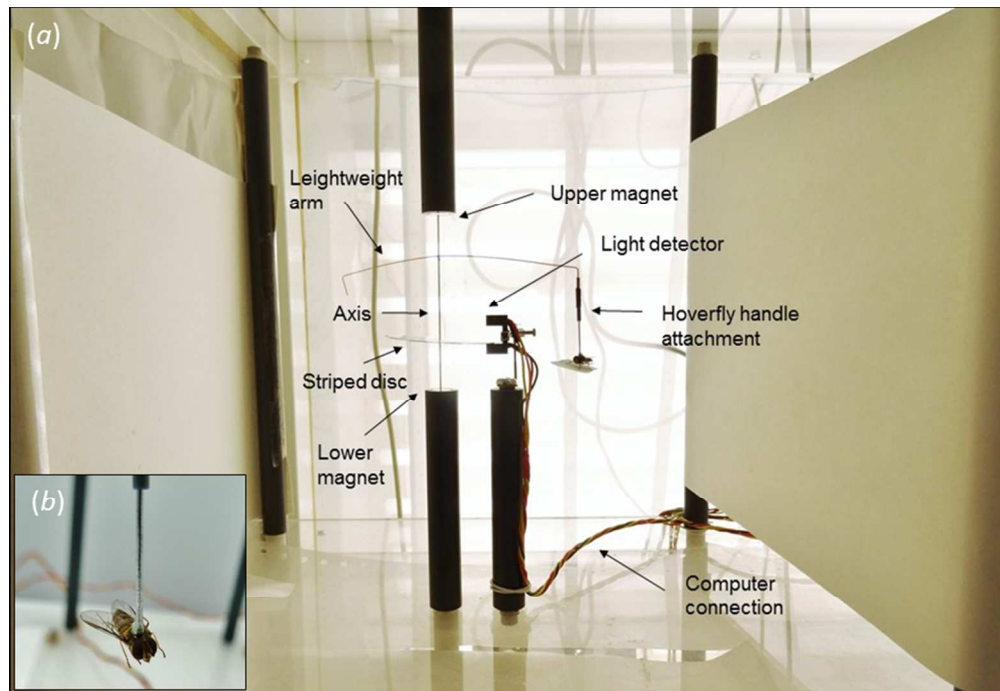


Figure 1. Tethered flight mill. Labelled photograph of an individual flight mill (a) and a close up of *Episyrphus balteatus* attached to the flight mill (b).

160x110mm (150 x 150 DPI)

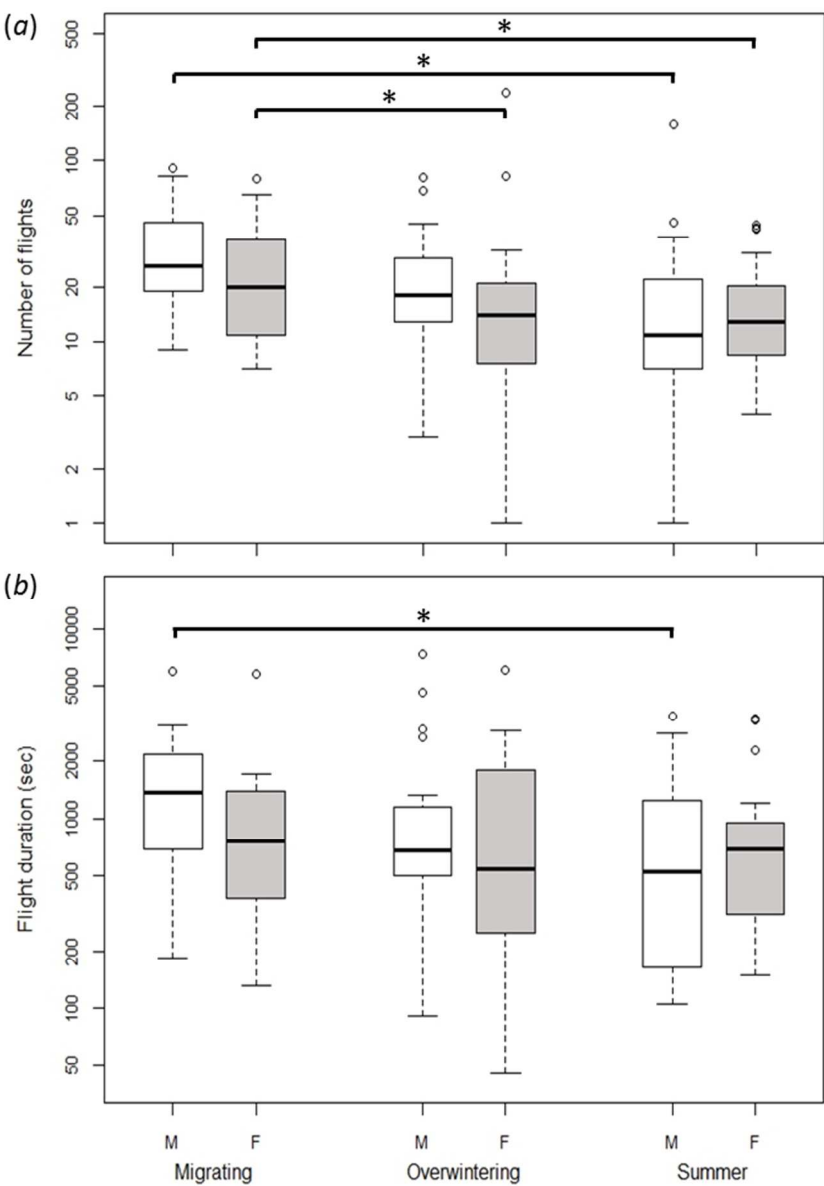


Figure 2. Number of flights (a) and flight duration in seconds (b) between phenotypes and sexes of first generation hoverflies. For clarification, only significant p-values are indicated in the graph. *p < 0.05. Number of flights and flight duration have been log transformed.

122x166mm (150 x 150 DPI)

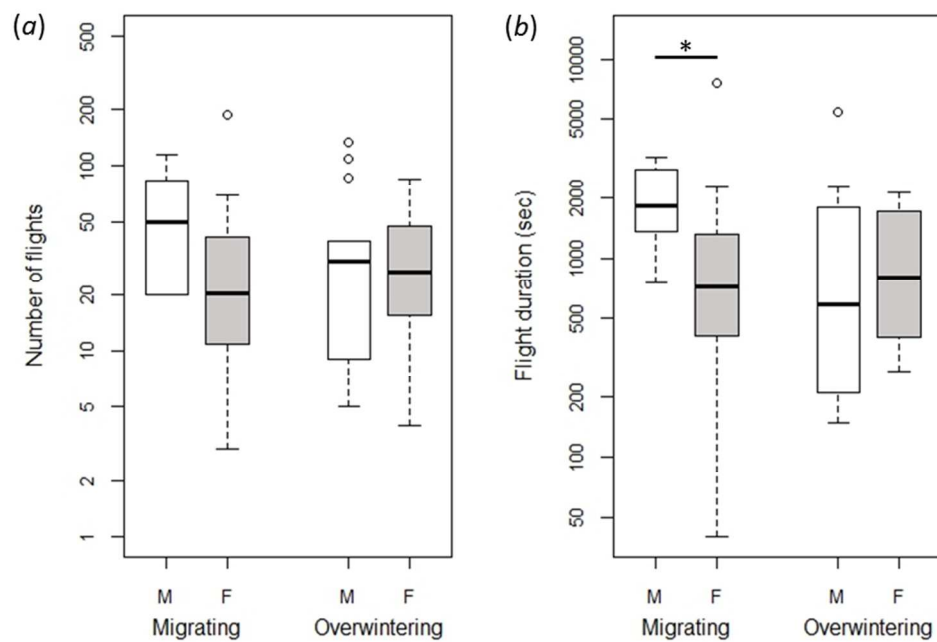


Figure 3. Number of flights (a) and total flight duration in seconds (b) between wild caught migrating and overwintering hoverflies. For clarification, only significant p-values are indicated in the graph. * $p < 0.05$. Number of flights and flight duration have been log transformed.

163x109mm (150 x 150 DPI)